Restoring CFTR Function Reduces Airway Bacteria and Inflammation in People With Cystic Fibrosis and Chronic Lung Infections

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ONLINE DATA SUPPLEMENT

SUPPLEMENTAL METHODS

Cohort Characteristics

All study visits were performed at National Referral Centre for Adult Cystic Fibrosis at St. Vincent's University Hospital, Dublin, Ireland, where all subjects (people with CF and *CFTR*-G551D mutations) received their routine CF care. Subjects' median age was 29.5 years (range 22-57), and 9 of 12 subjects were female. Subjects manifested a range of lung dysfunction; FEV_1 % predicted ranged from 34-101, with a mean FEV_1 % predicted of 64.2.

Written informed consent was obtained from all study participants following Research Ethics Committee approval. Inclusion criteria consisted of: age 18 years or older, clinical diagnosis of CF with the *G551D-CFTR* mutation on at least one allele (any known or unknown mutations allowed in second allele), stable clinical status with no significant changes in health status in the 14 days prior to day 0 visit, and written informed consent. Subjects were excluded if they (a) participated in the VX-770 Extended Access Program or had used of ivacaftor within 6 months prior to the day 0 visit, (b) exhibited any upper or lower respiratory symptoms requiring treatment with oral, inhaled or IV antibiotics within the 2 weeks prior to the day 0 visit, (c) had a history of solid organ transplantation, (d) pregnant or breast-feeding, or (e) exhibited the a condition or abnormality that in the opinion of the investigator would compromise the safety of the subject or the quality of the data. Subjects were allowed to continue standard therapy for CF except for treatments noted in the exclusion criteria described above.

All subjects began taking ivacaftor on March 4th, 5th or 8th, 2013, when ivacaftor became available in Ireland. At days 0, 2 and 7, each subject provided a spontaneously expectorated sputum sample and a blood sample, and underwent pulmonary function testing and sweat chloride testing. On day 0, subjects also underwent whole lung CT scanning. At later time points, only spontaneously expectorated sputum samples were obtained. Follow up pulmonary function testing was performed as part of subjects' routine clinic care, and repeat CT scans were performed at 1 year of treatment on 7 subjects. Sputum specimens were obtained from all 8 *P. aeruginosa*-positive subjects at early time points, and at least 6 of the *P. aeruginosa*-positive 8 subjects at each later time point.

Sweat Chloride Measurements. Sweat was collected with the Macroduct® collection system (Wescor, Logan UT), and sweat chloride levels were measured using routine standard laboratory techniques.

Spirometry. Spirometric measurements were obtained in accordance with the American Thoracic Society Standards (25). The forced expiratory volume in one second (FEV_1) percent predicted values are based on the ECCS 1993 values for adults.

Sputum processing for quantitation of bacterial density and inflammatory biomarkers, determination of microbiota relative abundance, and sputum proteomics. Sputum specimens were homogenized with cold 0.1% dithiotheitol (DTT). One aliquot of homogenized sputum was immediately frozen at -80^{o}C. This specimen was later used for genomic DNA isolation, followed by quantitative PCR and total 16S rRNA gene sequencing (see below). A

second aliquot of homogenized sputum was serially diluted, and plated on MacConkey agar to quantify *P. aeruginosa* counts. Homogenized sputum aliquots were also processed for quantification of sputum inflammatory markers (see below), and for mass spectroscopy to characterize sputum supernatant proteome (see below).

Bacterial DNA extraction.

DNA was extracted from DTT-treated sputum using the MO BIO PowerSoil DNA Isolation Kit with modifications to optimize DNA yield. Briefly; DTT-treated sputum was thawed on ice and homogenized for 30 seconds by repeated mixing using a 1 mL pipette. A 125 μ i aliquot of the homogenate was transferred to a sterile 1.5 ml microfuge tube, to which 20 μ a of lysozyme (final concentration 2.9mg/ml) and 20 μ o of lysostaphin (final concentration of 0.14 mg/ml) were added. The specimen was mixed gently and incubated at 37°C for 30 minutes with gentle rocking. The mixture was then transferred to a sterile 2-ml screw cap tube containing 0.5 g of sterile DNA free zirconia/silica beads, 750 μ (of Power Bead lysis solution (MO BIO) and 60 μ s of solution C1 (MO BIO). The tube was incubated at 70°C for 10 minutes, then agitated at full speed for 90 seconds on a Mini-Beadbeater-16 (Biospec). All remaining steps were carried out as described by the manufacturer of the MO BIO PowerSoil DNA Isolation kit. The purified DNA sample was stored at -80°C.

Real-time quantitative PCR.

Quantitative PCR (qPCR) assays were used to quantify the total bacterial load (genome equivalents per gram of sputum (GE/g)), and the load (GE/g) of specific bacterial taxa in each sputum samples, including: *P. aeruginosa*, *Prevotella* spp., *Streptococcus* spp., and

Staphylococcus aureus. For *P. aeruginosa*, *Prevotella* spp., and *S. aureus*, probe-based 20 μ r qPCR reactions included 10 µl of iTaq Universal Probes master-mix (Biorad), 300 nM of each primer/150nM of probe (appropriate for each specific assay), and 1 μ L of template DNA; the remaining volume was made up with sterile nuclease-free water (see Table S16 for primer and probe sequences). The qPCR reaction was performed on a CFX96 Touch real-time PCR detection system (Biorad) using the reaction conditions as follows: initial incubation at 95ºC for 4 min, followed by 39 cycles of 95ºC for 10 seconds and 60ºC for 1 m. Quantification of *Streptococcus* genera was carried out using the iTaq™ Universal SYBR® Green Supermix assay. The 20 μ L qPCR reaction included 10 μ r of iTaq™ Universal SYBR® Green Supermix, 300nM of each primer (*tuf)*, and 1 μ M of DNA template; the remaining volume was reached using sterile nuclease-free water. The qPCR was performed on a CFX96 Touch real-time PCR detection system (Biorad). The reaction conditions were as follows: an initial incubation at 95ºC for 4 min, followed by 39 cycles of 95ºC for 10 seconds and 60ºC for 30 sec.

Standard curves were generated for each qPCR assay by growing the type culture strain of the representative bacteria (Table S17), then isolating the DNA as described earlier. The DNA concentration for each sample was measured using the Qubit dsDNA assay kit (Life Technologies), using the DNA concentration and the mass of a single genome of the bacteria used for the standard curve the number of genome equivalents (GE) present in the extracted sample could be calculated. The DNA was serially diluted to create the standard curve for each qPCR assay.

Bacterial 16S rRNA gene sequencing and analyses.

The V3-V5 variable region of the 16S rRNA gene was amplified from each sample with primers containing Illumina adapter sequences, and a 12-bp index sequence for sample multiplexing (Table S18). Triplicate PCRs were performed for each sample in a total 25 μ . reaction volume containing 1x ThermoPol® reaction buffer (NEB), 10 μ of each primer, 2.5U of taq DNA polymerase (NEB), 2 μ D of DNA template and 18.5 μ o of molecular grade water using the following reaction conditions: an initial incubation at 94°C for 5 minute followed by 30 cycles of 94°C for 30 seconds, 55°C for 1 minute, and then 72°C for 1 m 30 seconds. After PCR the triplicate amplicons for each sample were pooled. Amplicon concentration of each pool was measured using the Qubit dsDNA assay kit (Life Technologies). Sequencing libraries for each sample were pooled in equal concentrations before sequencing for 600 cycles on an Illumina MiSeq desktop sequencer using the Miseq Reagent Kit v3. The 300 bp read 1 sequencing reads for each sample were de-multiplexed using the MiSeq Control Software. De-multiplexed sequencing reads were assigned to operational taxonomic units (OTUs) by UClust searching against the Silva rRNA gene database using Qiime (26, 27). Shannon alpha diversity was calculated using Qiime (27).

Quantification of sputum inflammatory biomarkers. Sputum was processed according to the CF Therapeutics Development Network Coordinating Center standard operating procedure. Briefly, DTT-treated aliquots of sputum were centrifuged at 250xg for 10 min at 4^{o}C, pellets were recovered for proteomic analysis (see below), and supernatants were then centrifuged at 4000xg for 20 min at 4^{o}C. Supernatants from the second spin were either frozen directly at -80^{o}C until analysis, or treated with protease inhibitor cocktail (Roche) and then frozen. Specimens were analyzed as previously described for interleukin (IL)-8, IL-6, and IL-1L [Luminex

Multiplex Bead, R&D Systems; Abingdon, Oxon, UK] and free neutrophil elastase (NE) activity [Spectrophotometric assay, Sigma Diagnostics; St. Louis, MO]. The lower limits of detection for these assays were: IL-6, 0.95 pg/ml; IL-8, 0.78 pg/mL; IL-1β, 0.34 pg/ml; free elastase , 0.25 μg/mL.

Proteomic Analysis of host proteins in sputum fractions.

***Proteome sample preparations*:** For sputum supernatant fractions, homogenized sputum was digested with Hyclone trypsin (1/10 volume dilution), incubated at 37^{o}C for 20 minutes, and then centrifuged at 100xg for 10min at 4^{o}C. The supernatant from this slow spin was recovered and centrifuged at 10,000xg for 20min at 4^{o}C. Supernatants recovered from the fast spin were frozen for proteomic analysis.

Proteins were extracted from sputum samples with lysis buffer [100 mM Tris (pH 8.0), 4 % SDS, 10 mM DTT]. Extraction mixtures were incubated at 95^{o}C heat block for 5 min. SDS was removed through buffer exchange with Amicon filters (10 KDa MWCO) (Millipore, Billerica, MA, USA) to urea buffer [50 mM NH_4HCO_3, 8 M Urea]. Proteins were alkylated, and digested sequencing grade Trypsin (Promega, Madison, WI, USA) overnight, using enzyme to substrate ratio (1:50, μg: μg). Tryptic peptides were purified with C18 Sep-Pak columns (Waters, Milford, Mam USA), dried, and resuspended with 0.1% formic acid for LC-MS/MS analysis.

***LC-MS/MS analysis*:** Proteome samples were analyzed with a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) coupled to an EASY-nLC 1000 system (Thermo Fisher Scientific). Reversed-phase separation was performed with a 3 cm trap

column (packed in-house with 200 Å C18 magic bead, 5 µm) (Bruker, Billerica, MA, USA) and a 65 cm analytical column (100 Å C18 magic bead, 5 µm), using mobile phase of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). A linear separation gradient with 2%-10% solvent B for 1 min, 10%-30% solvent B for 89 min, and 30%-80% solvent B for 1 min was used. The flow rate was set to 300 nL/min.

Data dependent acquisition (DDA) analysis with Q Exactive Plus included MS (28) analysis with scan range 400-2000 m/z, scan resolution 70,000 and automatic gain control (AGC) target value 1×10^6, and MS/MS analysis for the top 20 most abundant precursor ions detected in MS^1 scans. MS/MS analysis included isolation window 1.6 m/z, fragmentation energy 25 NCE, scan resolution 17,500, AGC target value 5×10^4. Charge exclusion for 1 + and unassigned ions was enabled. Dynamic exclusion of 30 s was used. Each sputum sample was analyzed with three LC-MS/MS replicate analyses.

***Proteome identification and quantitation*:** The raw data was converted to mzXML files with ReAdW (version 4.2.1). The mzXML files were searched with Comet (28) (version 2015.01 rev.02) against the concatenated database containing forward and reverse protein sequences of Human genome and *Pseudomonas aeruginosa PAO1* genome (total of 56,798 entries, downloaded from Uniprot (29) on May 7, 2014). Search parameters included 20 ppm precursor mass tolerance, ^{13}C offsets (-1/0/1/2/3) enabled, fragment bin tolerance 0.1, fragment bin offset 0, variable modifications of methionine oxidation (15.9949 Da), fixed modification of cysteine carbamidomethylation (57.021464 Da). Only fully tryptic peptide sequences were considered,

and up to 2 missed cleavages were allowed. The false discovery rate for peptide identification was determined with the target-decoy approach (30), and was controlled at 1%.

Label-free quantitation for MS^1 precursor ion intensity was performed with XPRESS (31). Peptide chromatograms (time, intensity) were used to determine peptide ratios. Only peptides that were quantified in at least two technical replicates, and with Coefficient of Variation (CV) of chromatogram intensity < 80% in technical replicates were used for quantitative comparison to other samples. The CV filtering process reduced the noise in peptide quantitation. Peptide ratios quantified with different charge states were averaged to represent the peptide ratios. The log2 of the peptide ratios were averaged to determine the log2 of protein ratios. The protein fold changes were obtained with the anti-log2 transformation of log2 protein ratios.

MLST analysis

We collected 96 *P. aeruginosa* isolates that grew from day 0 sputum samples, and the latest time point available (between 90-975 days depending on the subject), pooled the DNA from each sample, and sequenced the pools using Illumina technology. Consensus sequences for each subjects' isolates were generated (Fig S1) and then aligned to MLST sequences from the PubMLST Pseudomonas aeruginosa web site (http://pubmlst.org/ paeruginosa/) (14). Briefly, sequencing reads were mapped to set using BWA-MEM 0.7.13 (Li H. (2013) arXiv:1303.3997v1 [q-bio.GN]). Indexing and initial variant calls for these alignments were then performed with SAMtools 1.3.1 and BCFtools 1.3.1 (32). Alignments and variant calls were further refined using Broad Genome Analysis Toolkit 3.5 per the GATK Best Practices Pipeline (33). Consensus MLST sequences were called using the GATK FastaAlternateReferenceMaker. These were then

aligned to all MLST alleles with NCBI Blast 2.2.31+ (34). MLST sequence types were assigned based on the most homologous sequence identity and bitscore values. Plotting of MLST results was done with matplotlib (35).

High Resolution Computed Tomography

The scanning protocol was based on the Spiromics study protocol and other previously published studies (36, 37). Scans were preceded by three deep breaths to standardize lung volume history. Subjects were coached to reach TLC and RV. These maneuvers were rehearsed prior to scanning. All scans were performed as a helical acquisition at voltage of 120 kVp and tube current of 180 mA for inspiratory acquisition and tube current of 100 mA for expiratory acquisition on a GE HD750 CT scanner (General Electric Healthcare, USA). The slice thickness was 0.625 mm with an interval of 0.5 mm and a pitch of 0.984:1. Scans were reconstructed with filtered back projection and standard kernel, and they were de-identified prior to transfer to the University of Washington for analysis. For scoring, the radiologists applied each of the 5 parameters of the Brody score (bronchiectasis, peri-bronchial wall thickening, mucous plugging, parenchymal damage, and air trapping) to each of the 6 lobes of the lung (RUL, RML, RLL, LUL, Lingula, and LLL), and the sum of all scores was registered as the total Brody score.

Statistical Analyses

Bacterial, FEV_1 and diversity index slopes were estimated via piece- wise mixed effects linear regression models with a random effect for each subject's intercept and slope at study start and also immediately after initiating ivacaftor. These models included a fixed binary 'treatment' effect (pre versus post ivacaftor) that was tested within the confines of the model. An

unstructured correlation matrix was implemented, and means and 95% confidence intervals from the models were presented. Similarly, piecewise linear models were used to estimate slopes between Day 0 and 210 and after the change point Day 210 to Day 975. Tests for changes in slopes at Day 210 were performed using two-sided, alpha=0.05 tests and mean slope estimates are reported with 95% confidence intervals.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Multi-locus sequence typing of pooled *P. aeruginosa* isolates collected pre- and post ivacaftor. Ninety-six *P. aeruginosa* isolates were cultured from sputum samples collected at the indicated time points, isolates from each sample were pooled, and DNA from the pool was sequenced. Consensus sequence alignments were generated for each multi-locus sequence type (MLST) loci and the MLST type determined. Each MLST loci is color coded (*acs* = grey, *aro* = blue, *gua* = red, *mut* = orange, *nuo* = green, *pps* = purple, *trp* = teal), with different shades indicating different alleles at that locus. The * indicates subjects in whom the *acs* loci showed ambiguity.

Figure S2. Comparison of differences in population MLST allele frequency between before and after treatment *P. aeruginosa* pools from the same and different subjects. The percent change in MLST allele frequency (Y-axis) between two pools of isolates. Each position of the indicated MLST loci are represented on the X-axis. The left column shows differences in allele frequencies between pre- and post-ivacaftor pools of *P. aeruginosa* isolates from the same subjects. The right column shows differences in allele frequencies between pools of *P. aeruginosa* isolates from different subjects. Allele frequency changes were measured by

counting high-quality sequence read base alignments. To determine if there was a statistically significant difference in the change in allele frequency when comparing within-subject pools and between-subject pools, the following calculations were performed: the percent change at each base (if any) was summed for all MLST loci for each pair of pools. The results for each pair of pools were grouped into within-patient and between-patient categories, and these 2 categories were compared using the Students' t-test to generate a *p* value.

Figure S3. Pulsed-field gel electrophoresis (PFGE) analysis of *P. aeruginosa* from Subjects 4, 5, and 9 before ivacaftor after ivacaftor. The dendrogram indicating genetic similarity of the isolates is shown at the left portion of the figure and was generated using Bionumerics software with optimization and tolerance settings of 0.5% and 1.0%, respectively. The source patient and day of isolate culture ("Isolate") and the PFGE group number ("Group") are indicated next to each PFGE pattern. Clonality is defined as isolates at least 90% genetically similar. The *Salmonella* serotype *Braenderup* H9812 PFGE pattern was used for gel-to-gel normalization.

Figure S4. Combined relative abundance of *Streptococcus* and *Prevotella* in sputum. Includes all available samples for subjects with chronic *P. aeruginosa* infections, and day 0-7 samples for subjects without chronic *P. aeruginosa* infections. Values were calculated by summing the proportion of 16S rDNA reads mapping to *Streptococcus* and *Prevotella* at each time point. Table S3 shows linear regression analysis using the mixed model which indicates that the slope of the combined relative abundance of *Streptococcus* and *Prevotella* species was positive in the first year (p=0.003), and exhibited a significant decrease in slope (p=0.008) thereafter.

Figure S5. Microbiota changes in the 4 study subjects not chronically-infected with *P. aeruginosa*. A. Change in abundance of total bacteria as measured by quantitative PCR of 16S rDNA in sputum. Red line shows the median values of all 12 subjects, the blue line shows the median value of the *P. aeruginosa*-negative subjects, and the black lines show values for individual *P. aeruginosa*-negative subjects. **B and C.** Relative abundance of *Burkholderia* (**B**), and *Staphylococcus* species (**C**) during the first week of treatment. (**B**) shows the 2 subjects chronically infected with *Burkholderia*; and (**C**) shows the 4 subjects not infected with *P. aeruginosa*. Sputum cultures performed by the clinical microbiology laboratory for the 4 subjects shown in (**C**) were *P. aeruginosa* negative, and 3 of these subjects were *Staphylococcus* culture positive (by culture). **D.** Average changes in mean relative abundance of *Pseudomonas*, *Burkholderia*, and *Staphylococcus sp.* DNA in sputum of subjects in which each organism was detected.

Figure S6. Changes in sputum inflammation markers in the 4 study subjects not chronically-infected with *P. aeruginosa*. Changes in indicated inflammatory markers during the first week of ivacaftor treatment in the four patients without chronic *P. aeruginosa* infections. Red circles represent the average of all 12 patients (both the 8 *P. aeruginosa* positive and 4 *P. aeruginosa* negative patients). Blue circles represent the average of the 4 *P. aeruginosa* negative patients. White circles with black lines represent individual *P. aeruginosa* negative patients; *$p<0.05$.

Figure S1.

Subject	Day specimen collected	Determined Allelic profile/ sequence type	MLST loci						
			acs	*aro*	*gua*	*mut*	*nuo*	*pps*	*trp*
1	0	1394	11/6	5	6	3	74	13	7
	975	1394	11/6	5	6	3	74	13	7
2	0	1091	6	5	11	5	3	6	1
	975	1091	6	5	11	5	3	6	1
4*	0	526	19/92	29	1	3	1	15	7
	975	526	25/22	29	1	3	1	15	7
5*	0	526	19/92	29	1	3	1	15	7
	800	526	25/22	29	1	3	1	15	7
6	0	167	40/11	5	11	5	4	38	37
	975	167	40/11	5	11	5	4	38	37
7	0	395	148	5	1	1	1	12	1
	90	395	148	5	1	1	1	12	1
9*	0	1068	23	5	11	7	1	12	137
	975	1068	125/11	5	11	7	1	12	137
10	0	266	16	5	11	72	44	7	52
	975	266	16	5	11	72	44	7	52

Figure S2

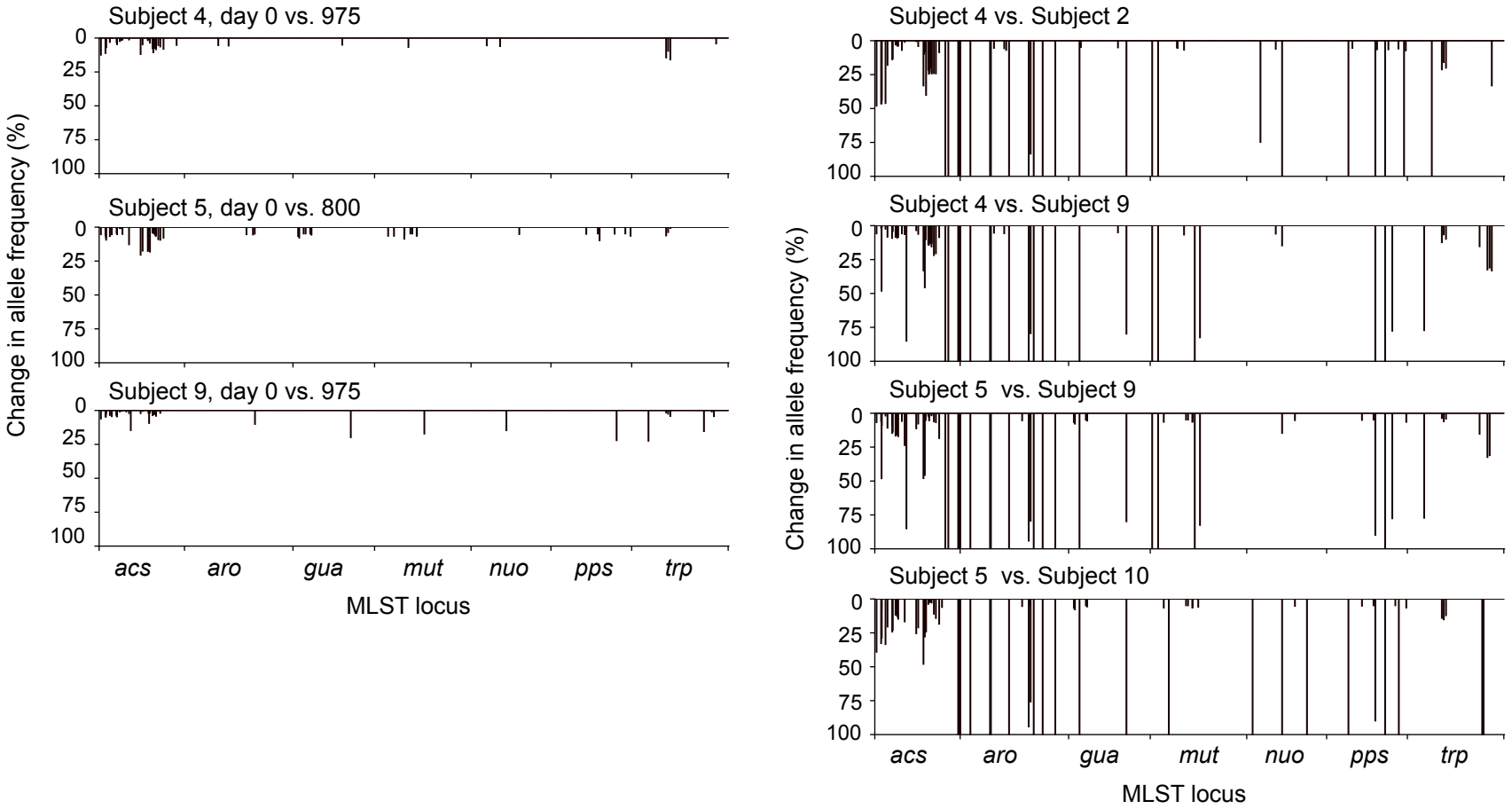
Subject 4, day 0 vs. 975
Subject 5, day 0 vs. 800
Subject 9, day 0 vs. 975
Subject 4 vs. Subject 2
Subject 4 vs. Subject 9
Subject 5 vs. Subject 9
Subject 5 vs. Subject 10
0
25
50
75
100
Change in allele frequency (%)
acs
aro
gua
mut
nuo
pps
trp
MLST locus

Figure S3

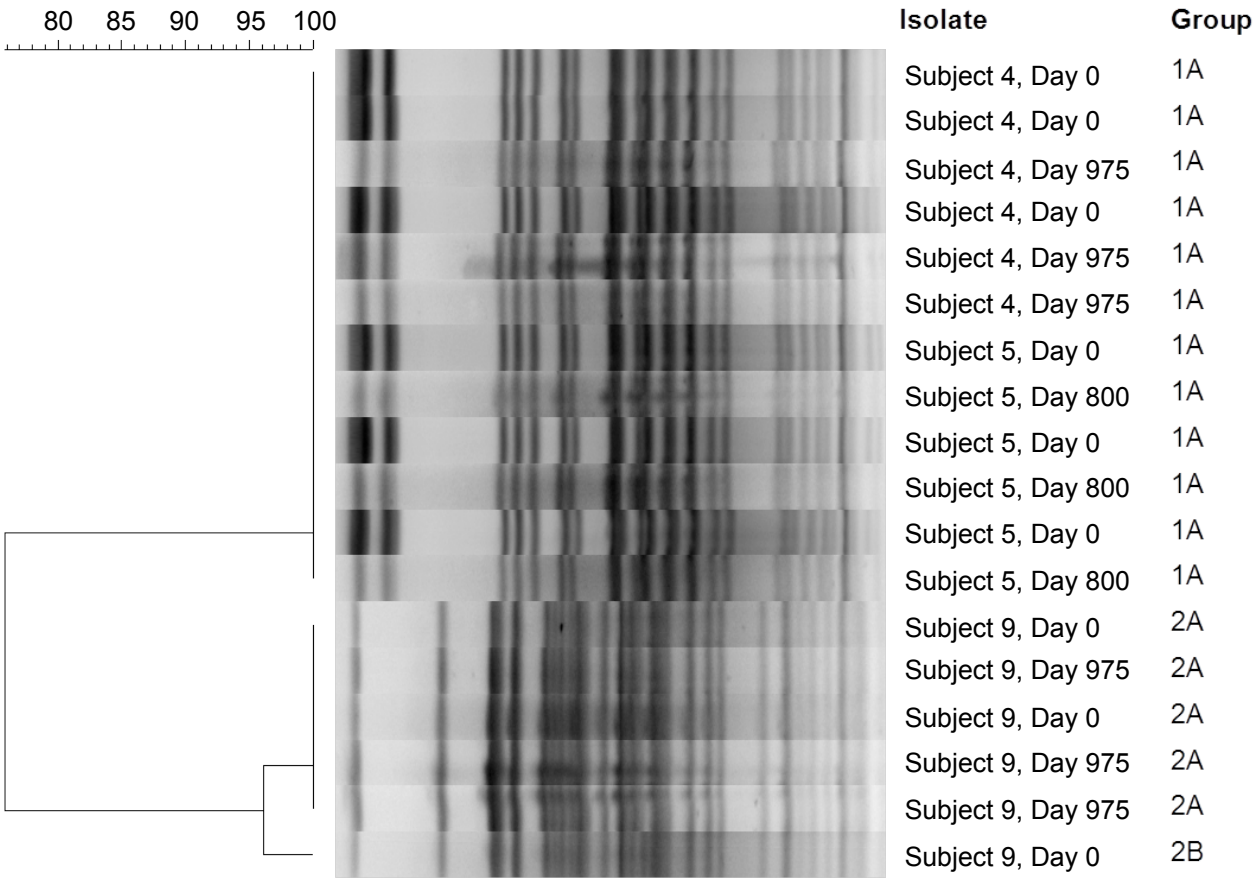
80
85
90
95
100
Isolate
Group
Subject 4, Day 0
1A
Subject 4, Day 0
1A
Subject 4, Day 975
1A
Subject 4, Day 0
1A
Subject 4, Day 975
1A
Subject 4, Day 975
1A
Subject 5, Day 0
1A
Subject 5, Day 800
1A
Subject 5, Day 0
1A
Subject 5, Day 800
1A
Subject 5, Day 0
1A
Subject 5, Day 800
1A
Subject 9, Day 0
2A
Subject 9, Day 975
2A
Subject 9, Day 0
2A
Subject 9, Day 975
2A
Subject 9, Day 975
2A
Subject 9, Day 0
2B

Figure S4

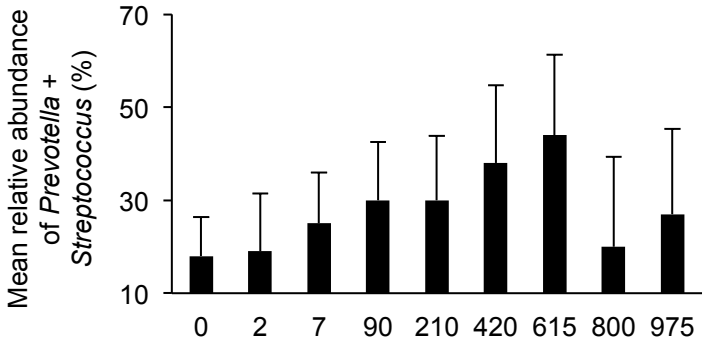
Mean relative abundance of *Prevotella* + *Streptococcus* (%)
70
50
30
10
0
2
7
90
210
420
615
800
975

Figure S5

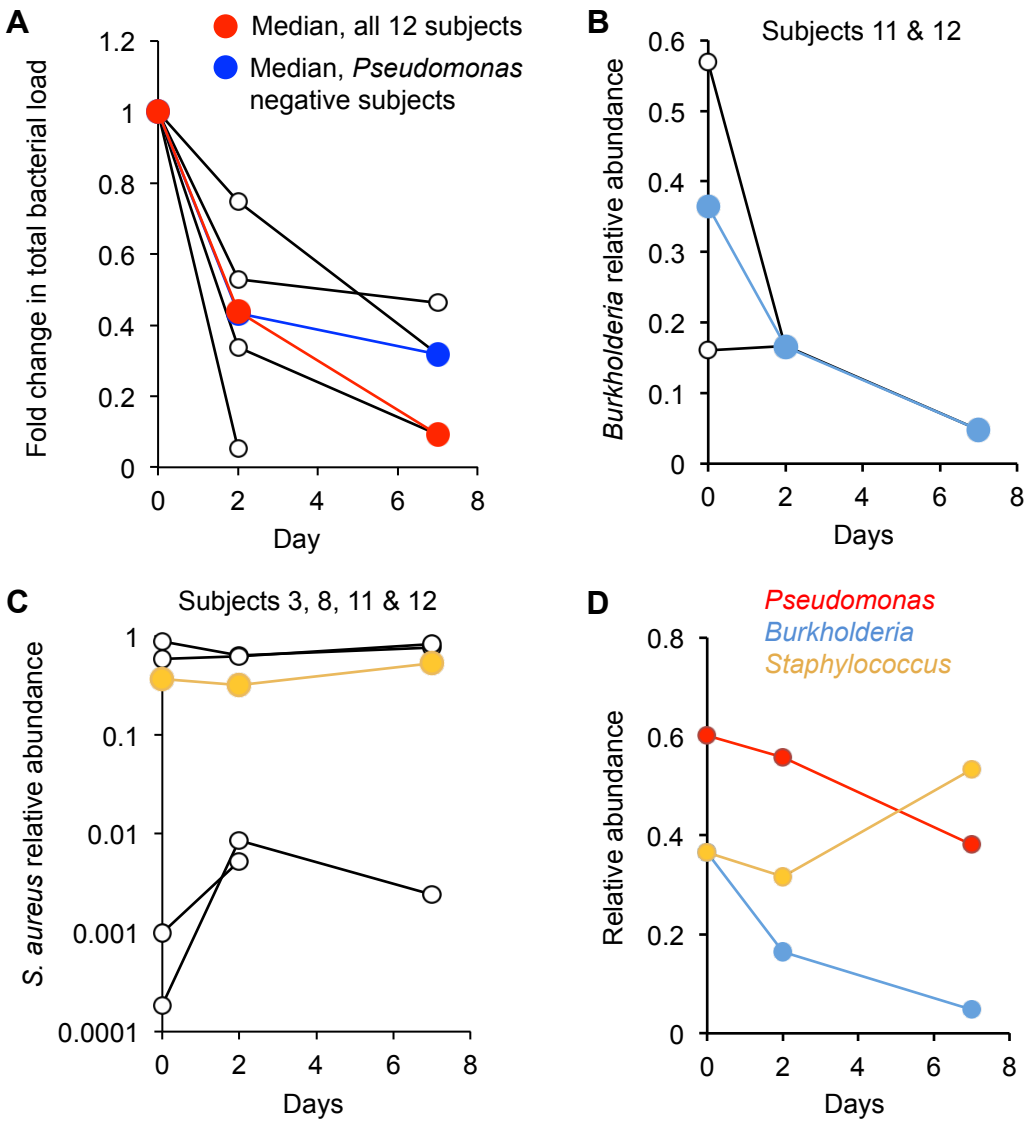
A
Median, all 12 subjects
Median, Pseudomonas negative subjects
Fold change in total bacterial load
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
Day
B
Subjects 11 & 12
Burkholderia relative abundance
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
Days
C
Subjects 3, 8, 11 & 12
S. aureus relative abundance
1
0.1
0.01
0.001
0.0001
0
2
4
6
8
Days
D
Pseudomonas
Burkholderia
Staphylococcus
Relative abundance
0.8
0.6
0.4
0.2
0
0
2
4
6
8
Days

Figure S6

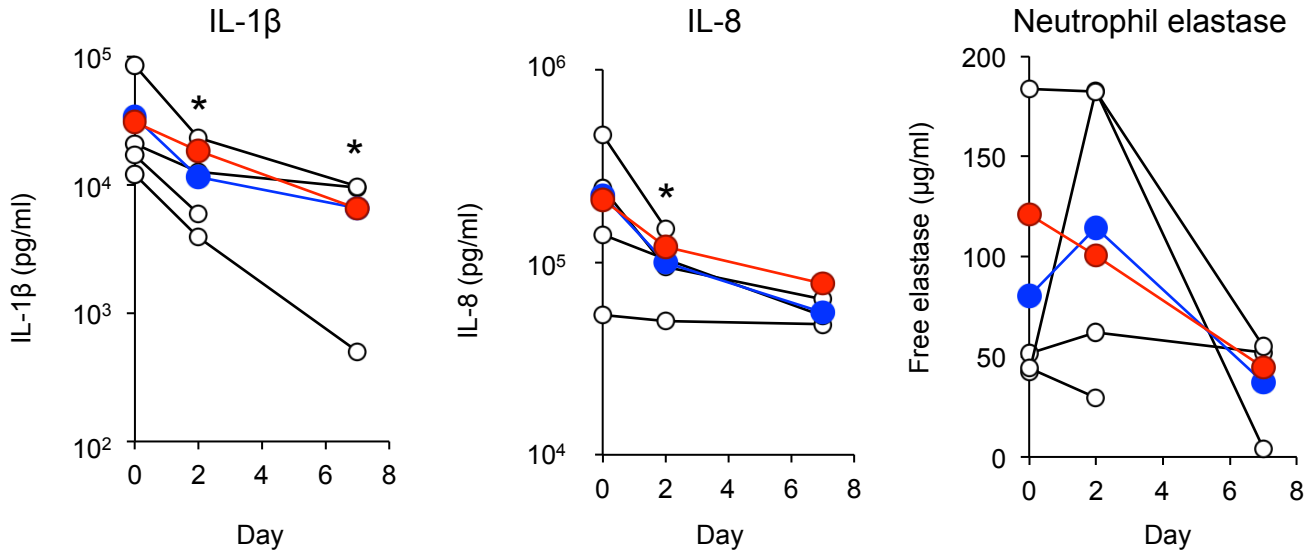
IL-1β
IL-1β (pg/ml)
10^5
10^4
10^3
10^2
*
*
0
2
4
6
8
Day
IL-8
IL-8 (pg/ml)
10^6
10^5
10^4
*
0
2
4
6
8
Day
Neutrophil elastase
Free elastase (μg/ml)
200
150
100
50
0
0
2
4
6
8
Day

Table S1. Sputum microbiology data from clinical cultures

Patient	Sputum cultures 2011 – March 2013	Sputum cultures March 2013 – June 2016	Notes
1	12/12 *P. aeruginosa* +	12/12 *P. aeruginosa* +	
2	5/5 *P. aeruginosa* + 5/5 *S. aureus* +	12/12 *P. aeruginosa* + 4/12 *S. aureus* +	Intermittent *S. aureus* + cultures post-ivacaftor
3	12/12 *S. aureus* +	11/12 *S. aureus* +	
4	4/4 *P. aeruginosa* +	3/3 *P. aeruginosa* +	
5	4/4 *P. aeruginosa* + 2/4 MRSA +	5/5 *P. aeruginosa* +	Intermittent MRSA + cultures pre-ivacaftor; none post
6	8/8 *P. aeruginosa* +	12/12 *P. aeruginosa* +	
7	5/5 *P. aeruginosa* + 1/5 *S. aureus* +	None available	Patient lost to follow up: no post-ivacaftor clinical cultures
8	4/4 *S. aureus* +	None available	Patient lost to follow up: no post-ivacaftor clinical cultures
9	14/14 *P. aeruginosa* +	13/13 *P. aeruginosa* +	
10	2/2 *P. aeruginosa* +	2/5 *P. aeruginosa* + 3/5 *S. aureus* +	*P. aeruginosa* negative in 2014 & 2015, but *P. aeruginosa*+ June 2016
11	5/5 *B. multivorans* + 5/5 *S. aureus* +	6/6 *B. multivorans* + 4/6 *S. aureus* + 1/6 *P. aeruginosa* +	*P. aeruginosa* + one time post-ivacaftor; subsequent culture was *P. aeruginosa* negative
12	2/2 *B. multivorans* +	2/2 *B. multivorans* + 2/2 *S. aureus* +	*S. aureus* detected post ivacaftor only

Table S2. Change in *P. aeruginosa* relative abundance and microbial diversity indices.

***P. aeruginosa* relative abundance**

	Estimate	Standard error	*p* value*	95% confidence interval
Slope day 0 to 210	-2.91	0.99	0.0046	-4.88 - -0.93
Slope day 210 to 975	0.082	0.35	0.82	-0.62 - 0.79
Change in slope at day 210	2.99	1.26	0.021	0.47 – 5.52

Richness

	Estimate	Standard error	*p* value*	95% confidence interval
Slope day 0 to 210	2.47	0.62	0.0002	1.23 – 3.70
Slope day 210 to 975	-0.42	0.22	0.061	-0.86 – 0.021
Change in slope at day 210	-2.88	0.79	0.0005	-4.46 – -1.31

Shannon Diversity

	Estimate	Standard error	*p* value*	95% confidence interval
Slope day 0 to 210	0.088	0.037	0.020	0.014 - 0.16
Slope day 210 to 975	-0.015	0.013	0.28	-0.041 – 0.012
Change in slope at day 210	-0.10	0.047	0.034	-0.20 - -0.0081

Evenness

	Estimate	Standard error	*p* value*	95% confidence interval
Slope day 0 to 210	0.016	0.0085	0.059	-0.00064 - 0.034
Slope day 210 to 975	-0.0026	0.0031	0.40	-0.0088 - 0.0036
Change in slope at day 210	-0.019	0.011	0.088	-0.041 - 0.0029

**p* values for slopes (day 0 – 210, or day 210 – 975) indicate significant difference in slope as compared to a slope of 0 (no change in the parameter). *p* values for change in slope indicate a significant change in slope following day 210.

Table S3. Change in relative abundance of *Prevotella* + *Streptococcus*

	Estimate	Standard error	*p* value*	95% confidence interval
Slope day 0 to 210	0.021	0.0067	0.0034	0.0072-0.034
Slope day 210 to 975	-0.0029	0.0024	0.22	-0.0077 - 0.0018
Change in slope at day 210	-0.024	0.0085	0.0079	-0.041 – -0.0065

**p* values for slopes (day 0 – 210, or day 210 – 975) indicate significant difference in slope as compared to a slope of 0 (no change in the parameter). *p* values for change in slope indicate a significant change in slope following day 210.

Table S4. Demographic and disease characteristics of subjects who did and did not present for CT Scans.

	Subjects who presented for CT scans *(n = 7)*	Subjects who did not present for CT scans *(n = 5)*	*p* value
Number *P. aeruginosa* + (%)	5 (71%)	3 (60%)	0.72
Number male (%)	1 (14%)	2 (40%)	0.40
Median age (years) (range)	27 (22 – 35)	33 (25- 57)	0.22
Baseline sweat chloride (mM) (± standard deviation)	94.8 (±14.7)	92.1 (± 20.3)	0.81
Mean % change in sweat chloride at day 2 ivacaftor (± standard deviation)	56.6% (±17)	49.0% (±16.6)	0.46
Mean baseline FEV_1 % predicted (± standard deviation)	57.7 (± 20)	73.4 (± 25)	0.28
Mean increase in % predicted FEV1 at day 7 ivacaftor (± standard deviation)	13 (± 8.6)	15 (± 8.6)	0.70

Table S5. Changes in Brody scores of high-resolution CT scans after one year of ivacaftor (N = 7).

	Pre-ivacaftor Mean *(Range)*	1 year post-ivacaftor Mean *(Range)*	Mean change *(95% CI)*	p value*
Total Brody Score	73.0 *(43 – 134.5)*	61.4 *(20.5 – 122.5)*	-11.6 *(-25.5 – 2.3)*	0.120
Bronchiectasis	20.8 *(5.75 – 47.5)*	18.7 *(5 – 46.25)*	-2.1 *(-9.6 – 5.3)*	0.762
Mucous Plugging	11.1 *(6 – 16)*	9.3 *(4 – 16)*	-1.9 *(-2.9 – -0.8)*	0.013
Peribronchial thickening	21.5 *(12 – 44)*	17.3 *(6 – 37.75)*	-4.2 *(-10.0 – 1.5)*	0.091
Parenchymal score	4.1 *(0 – 11)*	3.4 *(1 – 8)*	-0.7 *(-3.4 – 2.0)*	0.468
Hyperventilation/ air trapping	15.2 *(2 -22.5)*	12.1 *(0 – 22.5)*	-3.1 *(-6.6 – 0.3)*	0.130

* p values were calculated for log10 converted data, to account for wide spread of values and small cohort.

Table S6. Correlations between changes in sweat chloride at day 2 & 7 with reduction in microbiology at day 2 and 7.
Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Decrease in sweat chloride between days 0 and 2	Decrease in sweat chloride between days 0 and 7
Fold decrease in *P. aeruginosa* at day 2 (CFUs)	0.524 0.183 8	0.393 0.383 7
Fold decrease in *P. aeruginosa* at day 7 (CFUs)	0.667 0.0710 8	0.429 0.337 7
Fold decrease in *P. aeruginosa* at day 2 (qPCR)	0.286 0.493 8	0.357 0.432 7
Fold decrease in *P. aeruginosa* at day 7 (qPCR)	0.595 0.120 8	0.536 0.215 7
Fold decrease in total bacteria at day 2 (qPCR)	0.441 0.152 12	0.627 0.0388 11
Fold decrease in total bacteria at day 7 (qPCR)	0.682 0.0208 12	0.733 0.0158 11

Table S7. Correlations between changes in sweat chloride at day 2 & 7 with reduction in inflammatory parameters at day 2 and 7. Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Decrease in sweat chloride between days 0 and 2	Decrease in sweat chloride between days 0 and 7
Fold decrease in IL-1β at day 2 (Day 2/Day 0)	-0.524 0.0800 12	0.100 0.770 11
Fold decrease in IL-1β at day 7 (Day 7/Day 0)	0.218 0.519 11	0.697 0.0251 10
Fold decrease in IL-8 at day 2 (Day 2/Day 0)	-0.0699 0.829 12	0.309 0.355 11
Fold decrease in IL-8 at day 7 (Day 7/Day 0)	0.445 0.170 11	0.661 0.0376 10
Fold decrease in neutrophil elastase at day 2 (Day 2/Day 0)	0.497 0.101 12	0.545 0.0827 11
Fold decrease in neutrophil elastase at day 7 (Day 7/Day 0)	0.364 0.272 11	0.600 0.0667 10

Table S8. Correlations between reduction in *P. aeruginosa* and total bacteria at day 7 with reduction in inflammatory markers at day 7. Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Absolute decrease in IL-1β at day 7	Fold decrease in IL-1β at day 7	Absolute decrease in IL-8 at day 7	Fold decrease in IL-8 at day 7	Absolute decrease in NE at day 7	Fold decrease in NE at day 7
Log absolute decrease in *P. aeruginosa* at day 7 (CFU)	0.571 0.180 7	0.857 0.0137 7	0.321 0.482 7	0.714 0.0713 7	0.571 0.180 7	0.750 0.0522 7
Log fold decrease in *P. aeruginosa* at day 7 (CFU)	0.595 0.120 8	0.619 0.102 8	0.524 0.183 8	0.738 0.0366 8	0.262 0.531 8	0.571 0.139 8
Log absolute decrease in *P. aeruginosa* at day 7 (qPCR)	0.679 0.0938 7	0.929 0.0025 7	0.500 0.253 7	0.714 0.0713 7	0.786 0.0362 7	0.786 0.0362 7
Log fold decrease in *P. aeruginosa* at day 7 (qPCR)	0.548 0.160 8	0.571 0.139 8	0.167 0.693 8	0.286 0.493 8	0.119 0.779 8	0.286 0.493 8
Log absolute decrease in total bacteria at day 7 (qPCR)	0.555 0.0767 11	0.618 0.0426 11	0.582 0.0604 11	0.691 0.0186 11	0.682 0.0208 11	0.645 0.0320 11
Log fold decrease in total bacteria at day 7 (qPCR)	0.255 0.450 11	0.409 0.212 11	0.264 0.433 11	0.327 0.326 11	0.245 0.467 11	0.264 0.433 11

Table S9. Correlations between changes in microbiology parameters at day 2 & 7 with improvements in lung function at day 2 and 7. Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Absolute increase in FEV_1 Day 2	Absolute increase in FEV_1 Day 7	Relative increase in FEV_1 Day 2	Relative increase in FEV_1 Day 7	Absolute increase in FEV_1 % predicted Day 2	Absolute increase in FEV_1 % predicted Day 7
Fold decrease in *P. aeruginosa* at day 2 (CFUs)	-0.214 0.610 8	-0.0599 0.888 8	-0.500 0.207 8	-0.143 0.736 8	-0.132 0.756 8	-0.0362 0.932 8
Fold decrease in *P. aeruginosa* at day 2 (qPCR)	-0.524 0.183 8	-0.0958 0.822 8	-0.619 0.102 8	-0.286 0.493 8	-0.419 0.301 8	-0.120 0.776 8
Fold decrease in total bacteria at day 2 (qPCR)	-0.706 0.0102 12	-0.189 0.556 12	-0.792 0.0022 12	-0.399 0.199 12	-0.719 0.0084 12	-0.260 0.415 12
Decrease in *P. aeruginosa* relative abundance at day 2	-0.310 0.456 8	-0.850 0.0075 8	-0.190 0.651 8	-0.690 0.0580 8	-0.192 0.649 8	-0.880 0.0040 8
Fold decrease in *P. aeruginosa* at day 7 (CFUs)	-0.405 0.320 8	-0.120 0.778 8	-0.571 0.139 8	-0.190 0.651 8	-0.419 0.301 8	-0.108 0.798 8
Fold decrease in *P. aeruginosa* at day 7 (qPCR)	-0.524 0.183 8	-0.0240 0.955 8	-0.524 0.183 8	-0.262 0.531 8	-0.611 0.108 8	0.000 1.00 8
Fold decrease in total bacteria at day 7 (qPCR)	-0.682 0.0208 11	-0.0592 0.863 11	-0.733 0.0102 11	-0.291 0.386 11	-0.836 0.0014 11	-0.128 0.708 11
Decrease in *P. aeruginosa* relative abundance at day 7	-0.357 0.385 8	-0.467 0.243 8	-0.429 0.289 8	-0.452 0.260 8	-0.443 0.272 8	-0.494 0.213 8

Table S10. Correlations between reduction in *P. aeruginosa* at day 7 with reduction in inflammatory markers at day 420.
Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Absolute decrease in IL-1β at day 420	Fold decrease in IL-1β at day 420	Absolute decrease in IL-8 at day 420	Fold decrease in IL-8 at day 420	Absolute decrease in NE at day 420	Fold decrease in NE at day 420
Log absolute decrease in *P. aeruginosa* at day 7 (CFU)	0.300 0.624 5	0.300 0.624 5	-0.200 0.747 5	0.200 0.747 5	0.500 0.391 5	0.900 0.0374 5
Log fold decrease in *P. aeruginosa* at day 7 (CFU)	0.600 0.208 6	0.314 0.544 6	0.543 0.266 6	0.714 0.111 6	0.257 0.623 6	0.657 0.156 6
Log *P. aeruginosa* counts (CFUs) at day 7	-0.429 0.397 6	0.200 0.704 6	-0.771 0.0724 6	-0.829 0.0416 6	0.200 0.704 6	0.0857 0.872 6
Log absolute decrease in *P. aeruginosa* at day 7 (qPCR)	0.300 0.624 5	0.700 0.188 5	0.300 0.624 5	-0.300 0.624 5	0.600 0.285 5	0.600 0.285 5
Log fold decrease in *P. aeruginosa* at day 7 (qPCR)	0.600 0.208 6	0.314 0.544 6	0.543 0.266 6	0.714 0.111 6	0.257 0.623 6	0.657 0.156 6
Log *P. aeruginosa* counts (qPCR) at day 7	-0.429 0.397 6	-0.0286 0.957 6	-0.657 0.156 6	-0.829 0.0416 6	-0.0857 0.872 6	-0.371 0.469 6

Table S11. Correlations between reduction in P. *aeruginosa* and total bacteria at day 420 with long term improvements in lung function. Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Absolute increase in FEV_1 Day 0 - 420	Absolute increase in FEV_1 Day 7- 420	Relative increase in FEV_1 Day 0 - 420	Relative increase in FEV_1 Day 7 - 420	Change in FEV_1 slope (L/month)	Change in FEV_1 slope (% pred/month)
Fold decrease in *P. aeruginosa* at day 420 (CFUs)	-0.0286 0.957 6	-0.0857 0.872 6	-0.143 0.787 6	-0.200 0.704 6	-0.943 0.0048 6	-0.771 0.0724 6
Fold decrease in *P. aeruginosa* at day 420 (qPCR)	0.371 0.469 6	0.714 0.111 6	0.314 0.544 6	0.771 0.0724 6	-0.200 0.704 6	0.143 0.787 6
Fold decrease in total bacteria at day 420 (qPCR)	0.257 0.623 6	0.200 0.704 6	-0.0857 0.872 6	0.543 0.266 6	0.0286 0.957 6	-0.0286 0.957 6
Decrease in *P. aeruginosa* relative abundance at day 420	-0.257 0.623 6	0.143 0.787 6	-0.257 0.623 6	0.143 0.787 6	-0.714 0.111 6	-0.314 0.544 6

Table S12. Correlations between reduction in P. *aeruginosa* and total bacteria at day 7 with long term improvements in lung function. Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Absolute increase in FEV_1 Day 0 - 420	Absolute increase in FEV_1 Day 7- 420	Relative increase in FEV_1 Day 0 - 420	Relative increase in FEV_1 Day 7 - 420	Change in FEV_1 slope (L/month)	Change in FEV_1 slope (% pred/month)
Fold decrease in *P. aeruginosa* at day 7 (CFUs)	-0.286 0.493 8	-0.167 0.693 8	-0.381 0.352 8	-0.0476 0.911 8	-0.643 0.0856 8	-0.548 0.160 8
Fold decrease in *P. aeruginosa* at day 7 (qPCR)	-0.262 0.531 8	-0.167 0.693 8	-0.333 0.420 8	-0.0952 0.823 8	-0.429 0.289 8	-0.310 0.456 8
Fold decrease in total bacteria at day 7 (qPCR)	-0.0636 0.853 11	0.000 1.00 11	-0.227 0.502 11	0.118 0.729 11	-0.155 0.650 11	-0.218 0.519 11
Decrease in *P. aeruginosa* relative abundance at day 7	-0.273 0.417 11	0.145 0.670 11	-0.0727 0.832 11	0.382 0.247 11	0.145 0.670 11	0.136 0.689 11

Table S13. Correlations between reduction in total bacteria at day 7 with reduction in inflammatory markers at day 420.
Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Absolute decrease in IL-1β at day 420	Fold decrease in IL-1β at day 420	Absolute decrease in IL-8 at day 420	Fold decrease in IL-8 at day 420	Absolute decrease in NE at day 420	Fold decrease in NE at day 420
Log absolute decrease in total bacteria at day 7 (qPCR)	-0.0857 0.872 6	0.143 0.787 6	-0.0286 0.957 6	0.257 0.623 6	-0.143 0.787 6	0.429 0.397 6
Log fold decrease in total bacteria at day 7 (qPCR)	0.257 0.623 6	0.0286 0.957 6	0.371 0.469 6	0.600 0.208 6	-0.200 0.704 6	0.371 0.469 6
Log total bacterial counts (qPCR) at day 7	-0.257 0.623 6	-0.0286 0.957 6	-0.771 0.0724 6	-0.886 0.0188 6	0.143 0.787 6	-0.0857 0.872 6

Table S14. Correlations between improvements in FEV_1 at days 2 and 7 with changes in diversity indices at days 2 and 7.
Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Absolute increase in FEV_1 Day 2	Absolute increase in FEV_1 Day 7	Relative increase in FEV_1 Day 2	Relative increase in FEV_1 Day 7	Absolute increase in FEV_1 % predicted Day 2	Absolute increase in FEV_1 % predicted Day 7
Absolute change in richness between day 0 to 2	0.277 0.383 12	0.364 0.245 12	0.246 0.441 12	0.340 0.279 12	0.255 0.423 12	0.391 0.209 12
Absolute change in Shannon diversity between day 0 to 2	0.0769 0.812 12	0.676 0.0158 12	0.0771 0.812 12	0.643 0.0240 12	0.158 0.624 12	0.702 0.0110 12
Absolute change in evenness between day 0 to 2	-0.00699 0.983 12	0.673 0.0166 12	0.0315 0.923 12	0.678 0.0153 12	0.0807 0.803 12	0.684 0.0141 12
Absolute change in richness Between day 0 to 7	0.364 0.272 11	0.105 0.759 11	0.346 0.297 11	0.318 0.340 11	0.470 0.144 11	0.119 0.728 11
Absolute change in Shannon diversity between day 0 to 7	0.136 0.689 11	0.506 0.113 11	0.342 0.304 11	0.736 0.0098 11	0.333 0.317 11	0.539 0.0872 11
Absolute change in evenness Between day 0 to 7	0.0182 0.958 11	0.633 0.0365 11	0.205 0.545 11	0.773 0.0053 11	0.205 0.544 11	0.676 0.0225 11

Table S15. Correlations between late and sustained improvements in FEV_1 with changes in diversity indices at days 7 and 420. Top = R, Middle = *p*-value, bottom = n. Red = $p < 0.05$, orange = $p < 0.1$.

	Absolute increase in FEV_1 Day 0 - 420	Absolute increase in FEV_1 Day 7- 420	Relative increase in FEV_1 Day 0 - 420	Relative increase in FEV_1 Day 7 - 420	Change in FEV_1 slope (L/month)	Change in FEV_1 slope (% pred/month)
Absolute change in richness Between day 0 to 7	-0.227 0.502 11	-0.255 0.450 11	-0.0364 0.916 11	-0.145 0.670 11	0.0546 0.873 11	-0.0546 0.873 11
Absolute change in Shannon diversity between day 0 to 7	0.0273 0.937 11	-0.245 0.467 11	0.164 0.631 11	-0.309 0.355 11	-0.218 0.519 11	-0.191 0.574 11
Absolute change in evenness Between day 0 to 7	0.155 0.650 11	-0.218 0.519 11	0.164 0.631 11	-0.318 0.340 11	-0.309 0.355 11	-0.282 0.401 11
Absolute change in richness Between day 0 to 420	0.600 0.208 6	0.429 0.397 6	0.429 0.397 6	0.657 0.156 6	0.200 0.704 6	-0.0286 0.957 6
Absolute change in Shannon diversity between day 0 to 420	0.314 0.544 6	-0.0857 0.872 6	0.200 0.704 6	0.143 0.787 6	0.429 0.397 6	-0.0857 0.872 6
Absolute change in evenness Between day 0 to 420	-0.0857 0.872 66	-0.486 0.329 6	-0.0857 0.872 6	-0.371 0.469 6	0.0857 0.872 6	-0.429 0.397

Table S16. Primers and probes for quantitative PCR assays

Target	Gene	Probe fluorophore	Direction	Sequence	Amplicon Size (bp)	Reference
All bacteria	*16S rRNA*		Forward	TCCTACGGGAGGCAGCAGT	466	Haarman
			Reverse	GGACTACCAGGGTATCTAATCCTGTT		
		FAM	Probe	CGTATTACCGCGGCTGCTGGCA		
P. aeruginosa	*gyrB*		Forward	CCTGACCATCCGTCGCCACAAC	221	Le Gall
			Reverse	CGCAGCAGGATGCCGACGCC		
		FAM	Probe	CCGTGGTGGTAGACCTGTTCCCAGACC		
Prevotella sp.	*16S rRNA*		Forward	CCAGCCAAGTAGCGTGCA	151	Martin
			Reverse	TGGACCTTCCGTATTACC		
		FAM	Probe	AATAAGGACCGGCTAATTCCGTGCCAG		
Streptococcus sp.	*tuf*^		Forward	GTACAGTTGCTTCAGGACGTATC	196	Picard
		No probe	Reverse	ACGTTCGATTTCATCACGTTG		
S.aureus	*nuc*		Forward	GCGATTGATGGTGATACGGTT	270	Maes, Brakstad
			Reverse	AGCCAAGCCTTGACGAACTAAAGC		
		FAM	Probe	GGTGTAGAGAAATATGGTCCTGAAGCAAGT		

^= SYBR Green Real-Time PCR assay

Table S16 references.

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Table S17. Bacterial taxa used in the construction of standard curves for taxa specific qPCR analysis.

Bacterial taxa	**Strain ID**
P. aeruginosa	PA01
P. melaninogenica	ATCC 25845
S. aureus	NCTC 8325
S. parasanguinis	ATCC 15911
S. salivarius	ATCC 7073

Table S18. Primers used for microbiota 16S rRNA library construction and sequencing

Primer name	Purpose	Sequence
Illumina-CCF	PCR	AATGATACGGCGACCACCGAGATCTACAC-**TATGGTAATTGT-***CCAGACTCCTACGGGAGGCAGC* Illumina adapter **Pad region** *CCF universal forward primer*
Illumina-Id1-926R	PCR	CAAGCAGAAGACGGCATACGAGAT-XXXXXXXXXXXX-**AGTCAGTCAGCC**-*CCGTCAATTYYTTTRAGTTT* Illumina adapter X Index X **Pad region** *926R universal reverse primer*
MiSeq-CCF	sequencing	**TATGGTAATTGT**-CCAGACTCCTACGGGAGGCAGC **Pad region** CCF universal forward primer
MiSeq-926R	sequencing	**AGTCAGTCAGCC**-CCGTCAATTYYTTTRAGTTT **Pad region** CCF universal forward primer
MiSeq-926R-Index	sequencing	AAACTYAAARRAATTGACGGGGCTGACTGACT